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Inhibitory Effect of Curcumin on Norovirus Infection and Its Mechanism

Curcumin을 이용한 항노로바이러스 효과 및 기전연구

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Abstract

Inhibitory Effect of Curcumin on Norovirus Infection and its Mechanism

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Norovirus outbreak is a major public health burden across the world. In immunocompromised hosts, norovirus can establish chronic infection. Chronically infected hosts have potential to act as a reservoir of emergent norovirus strain. The safe and affordable therapeutic agents to control chronic norovirus infection are required. Phytonutrients have been used as supplements in traditional medicine to enhance immune responses or resistance to infectious diseases. In this study, the effect of curcumin on chronic norovirus infection was evaluated using mouse model. C57BL/6 mice were orally inoculated with curcumin daily for 34 days and infected

with murine norovirus (MNoV) CR6 strain. Viral loads were quantified in stool and tissue samples by realtime PCR. Curcumin reduced MNoV in colon and mesenteric lymph nodes (MLN). Type I interferons (IFNs) were also enhanced in those tissues. Inhibition was not observed in ileum and peyer's patch (PP). Microbial community was assessed using 16S rRNA sequencing to understand tissue-specific MNoV inhibition. Family *Mogibacteriaceae* was changed significantly only in colon. Family *Mogibacteriaceae* abundance and virus quantity showed a weak negative correlation using correlation analysis. These results suggest that curcumin enhanced type I interferons in large intestines resulting in inhibition of MNoV. Specific enteric bacteria may be related to control MNoV.

Key words: Curcumin, Murine norovirus, Chronic infection, Microbiota

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I. Introduction

Norovirus outbreak is a major public health problem across the world (1). This highly infectious agent spreads person to person via a fecal-oral route (2). Though it causes acute gastroenteritis lasting 2 or 3 days and self-limited symptom (3), norovirus can establish chronic infection in immunocompromised hosts such as the elderly, malnourished individuals (4, 5). Chronically infected hosts have potential to act as a reservoir of emergent norovirus strain (6). The safe and inexpensive therapies to control chronic norovirus infection are required.

With the absence of small animal infection model for researching human norovirus (HuNoV), surrogate models such as murine noroviruses (MNoV) have been widely used (7). Controversies and limitations are always inherent how closely the effects on MNoV mimic HuNoVs. Any conclusions observed in MNoV model should be proven in HuNoV model. MNoV CR6 strain is reported to propagate in mouse persistently (8). Thus, this model is appropriate to evaluate effects of curcumin on chronic norovirus infection and analyze its mechanism.

For many years, a variety of phytonutrients have been used in therapeutic remedies to enhance immune responses or resistance to

infectious diseases. In this study, curcumin was used. Turmeric has been used an important ingredient of traditional medicines (9, 10). Curcumin, which is constituents of turmeric has been proven to show biological and pharmacological effects on a variety of human health problems (11). Curcumin was also shown to have antimicrobial and antiviral effects (12-14).

Researchers have screened phytonutrients on anti-noroviral effects. Flavonoids are found in most plant extracts that showed anti-noviral effects (15-17). Other phytonutrients, such as citric acid (18), chitosan (19), ginsenosides (20), and proanthocyanidins (21) were reported to anti-noroviral effects in *in vitro* cell-based tests. No study evaluating anti-noroviral effects of phytonutrients as a therapeutic agent in mouse model has been reported. It is more complex than treating to target cells directly at higher concentration in *in vitro* test.

In mouse models, the innate immune response plays a major role in limiting virus replication through interferon (IFN) pathway. MNoV gets accession to tissues through microfold (M) cells (22) which is a specialized cell in peyer's patch functioning antigen sampling from lumen and infects dendritic cells or macrophages (23). Type I and type II IFNs induced by antigen-presenting cells (APC) have been shown to

limit virus translation (24). According to recent studies, IFN-λ (type III interferon) induced by MNV.CW3 strain helped with virus clearance (25). In another study, depletion of microbiota by antibiotics made virus fail to propagate intestines and enhanced type III IFNs (26) suggesting that gut microbiota may related to innate immunity. Similarly, enteric bacteria have been reported to serve as an important factor for norovirus infection (26, 27).

In this study, we explored an anti-noroviral effect of curcumin using an animal infection model. We showed that curcumin enhanced innate immune responses in mice. Subsequently, microbial community change in tissues by curcumin was observed and a correlation between virus loads and specific bacteria abundance was assessed.

II. Materials and Methods

1. Viruses and reagents

Murine norovirus (MNoV) CR6 was kindly provided by Prof. Herbert Virgin (Washington University, St Louis, USA) and propagated in RAW264.7 cells maintained in Dulbecco's Minimal Essential Medium (DMEM) containing 10 % fetal bovine serum (Cyclone), 10 mM sodium bicarbonate (Gibco), 10 mM nonessential amino acids (Gibco), 10 mM HEPES (Gibco), and 50 μg/ml of gentamicin (Gibco). Infected cells were subjected to three times of freezing-thawing cycles. Then cultures were purified using chloroform and concentrated by centrifugation. Virus stocks were aliquoted and stored at -80 ℃ deep freezer. Curcumin was purchased from Sigma Aldrich (St.Louis, MO, USA)

2. Mice and infections

C57BL/6 (8 weeks old) female mice were purchased from Orient Bio Inc. (Seongnam, Korea) and housed at Seoul National University Hospital Biomedical Research Institute under an animal biosafety level 2 (ABL-2) specific-pathogen-free (SPF) conditions according to University guidelines. Animal protocols were approved by the Seoul National University Institutional Animal Care and Use Committees (IACUC). From a week prior to virus infection, mice were inoculated with a curcumin (500mg/kg/day) in 0.5% carboxymethyl cellulose (CMC, Sigma) everyday by oral route. To control group, vehicle was treated by orally. Infection was performed by orally with 200 μl of DMEM containing viruses (10^6 PFU). After infection, curcumin was inoculated for 27 days. Stools were collected at designated time points. Tissues were harvested and stored in RNAlater (Ambion, TX, USA) solution and frozen in a liquid nitrogen directly. Stools and tissues were stored at -80 °C.

3. Quantitative reverse transcription-PCR

Quantification of viral loads in stools and tissues was performed using Real-time PCR using ABI 7300 (Applied Biosystems) machine. Comparison of antiviral gene expression was performed using Rotor-Gene Q machine (Qiagen). Primers and probe which was used in this study listed in Table.1.

3.1 Virus quantification in stools

Stool samples were homogenized using a Vortex Adaptor (MOBIO Laboratories, Solana Beach, CA) with a PowerBead Tube (MOBIO) for 10min. Total RNA was extracted using the Quick-RNA Miniprep (Zymo Research, Irvine, CA) kit. MNoV from stool was measured using the AgPath One-step RT-PCR (Ambion, Life Technologies, Carlsbad, USA) kit (The reaction mixture was composed of 12.5 μl of 2X RT-PCR Buffer, 1 μl of 25X RT-PCR Enzyme Mix, 2.5 μl of template RNA, and 200nM primers and 100 nM probe). The cycling parameters were reverse transcription at 42°C for 30 min, initial denaturation at 95 °C for 10 min, 45 cycles of denaturation at 95 °C for 15s, annealing, and extension at 60 °C for 1 min.

3.2 Virus quantification in tissues

Tissue samples were homogenized PT-2000 E homogenizer

(POLYTRON). Total RNA from tissues was isolated with the Easy-spin Total RNA extraction kit (iNtRON, Korea) according to the manufacturer's protocol. 1 μg of tissue RNA was used for cDNA synthesis with the ImPromII reverse transcriptase system (Promega, Madison, WI). MNoV from tissues was measured using the TaqMan gene expression assays (Life Technologies, NY, USA) ribosomal protein S29 (RPS29) of house keeping gene was used for normalization. SYBR green quantitative PCR for RPS29 was performed using Power SYBR Green PCR Master Mix (Life technologies, CA, USA). For absolute quantification, serial dilutions of a plasmid including MNoV or RPS29 target sequence were used as a standard curve. Cycling parameters were identical to parameters used in MNoV quantification in stool assay with the exception of reverse transcription step.

3.3 Relative gene expression

For estimating expression levels of cytokine mRNA, Rotor-Gene SYBR Green PCR Kit (Qiagen) were used. GAPDH was used as an internal control. Cycling conditions were hold at 95°C for 5min and 40 cycles of denaturation at 95°C for 5 s, with annealing & extension for 10 s.

Table.1. Primer and probe sequences used in real-time PCR assay

Gene	Sequence
MNoV	F: CACGCCACCGATCTGTTCTG R: GCGCTGCGCCATCACTC P: 6FAM-CGCTTTGGAACAATG-MGBNFQ
RPS29	F: AGCAGCTCTACTGGAGTCACC R: AGGTCGCTTAGTCCAACTTAATG
IFN-α4	F: TGTGTGATGCAGGAACCTCCT R: GGTACACAGTGATCCTGTGG
IFN-β	F: ATAAGCAGCTCCAGCTCCAAG R: GTCTCATTCCACCCAGTGCTG
IFN-γ	F: TGAACGCTACACACTGCATC R: CGACTCCTTTTCCGCTTCCT
GAPDH	F: ATTGTCAGCAATGCATCCTG R: ATGGACTGTGGTCATGAGCC

F represents sequences of a forward primer

R represents sequences of a reverse primer

P represents sequences of a probe

4. DNA extraction and 16S rRNA sequencing

The gut microbiome was analyzed from mouse tissue samples (cecum, ileum, and colon). Cecum samples containing contents were homogenized using a Vortex Adaptor ((MOBIO Laboratories, Solana Beach, CA) with a PowerBead Tube (MOBIO) for 10min. Total DNA from cecum was extracted using the QIAamp FAST DNA Stool kit (Qiagen). Colon and ileum samples were homogenized using Mini-Beadbeater (Biospec Porudcts, Bartlesville, Oklar) with same bead tube used in cecum samples. DNA was extracted using G-spin Genomic DNA Extraction Kit (iNtRON, Korea).

16S rRNA genes from total DNA of tissues were amplified using the 515F and 806R primers specific for the V4 region, as described previously(28). The PCR products were run on an agarose gel to confirm and purified with MO BIO UltraClean PCR Clean-up Kit (MOBIO, Carlsbad, CA). After that, products were quantified using the KAPA Library Quantification Kit (KAPA Biosystems, Woburn, MA) and ABI 7300 (Applied Biosystems) machine. Every samples were pooled and sequenced on the Miseq (Illumina, San Diego, CA).

5. Sequence analysis

Sequences analyzed from Miseq were processed in QIIME platform (v1.8.0) (29). Sequence clustering was performed with 97% identity using a closed-method operational taxonomic unit (OTU) picking method. After alignment, taxonomy was assigned to each sample using the Ribosomal Database Project (RDP) classifier based on the Greengene database. Read counts from the OTU table were used for separating taxons to seven levels from kingdom to species and converted to relative abundance table for further analysis.

6. Bioinformatics and statistical analysis

LDA effect size (LEfSe) (30) analysis was performed to discover specific microbial biomarker and characterize statistically significant differences between groups. Web-based galaxy module was used for LEfSe analysis. In that process, the Kruskal-Wallis and Wilcoxon test were used and threshold of logarithmic LDA score was set to 3.0.

Non-metric dimensional scaling (NMDS) analysis, which is used for comparing microbial community between samples, was performed using the vegan package in R (31). The dissimilarity within samples was calculated using Bray-Curtis distance method.

The $2^{(-\Delta\Delta C_T)}$ method ($\Delta\Delta C_T=(C_{T,Target}-C_{T,Ref})_{treated}-(C_{T,Target}-C_{T,Ref})_{control}$) was used (where C_T is the threshold cycle) for calculating relative gene expression.

All data were analyzed with Prism 5 (GraphPad Software, San Diego, CA). Statistical significance was measured using Mann-Whitney test when comparing two groups, two-way ANOVA with Bonferroni post-tests comparing time course data. In all graphs, data were presented as mean ± sem. Statistical significance was given as * *P*-value <0.05, ** *P*-value < 0.01, *** *P*-value <0.001.

III. Results

1. Effect of curcumin on MNoV shedding in stool.

The effects of curcumin against murine norovirus replication were evaluated *in vivo* animal model. According to experiment schemata (Fig.1), curcumin (500mg/kg/day) was inoculated to mice everyday by oral gavage from 1 week before infection for boosting their effect. At 5 days post infection (DPI 5), stool samples were collected and analyzed by real-time RT-PCR. Virus shedding (3.02 ± 0.20 $\log_{10}$ copies/fecal) in stool from curcumin treated mice was reduced significantly comparing with control group (3.66 ± 0.18 $\log_{10}$ copies/fecal) (*p =0.0315) (Fig.2).

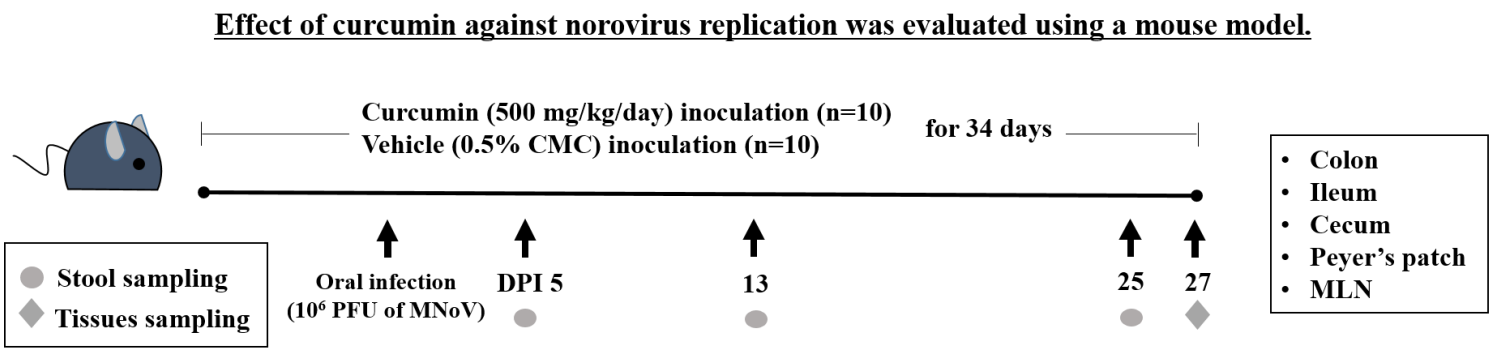

Figure 1. Mouse experiment schemata.

Mice were inoculated with curcumin and infected with MNoV orally. Curcumin (500mg/kg/day) or vehicle (0.5% CMC) was inoculated everyday from 1 week before infection to 27 DPI. Fecal samples were collected on 5, 13, and 25 DPI. Tissues were harvested on 27 DPI. DPI = days post infection; CMC (carboxymethylcellulose).

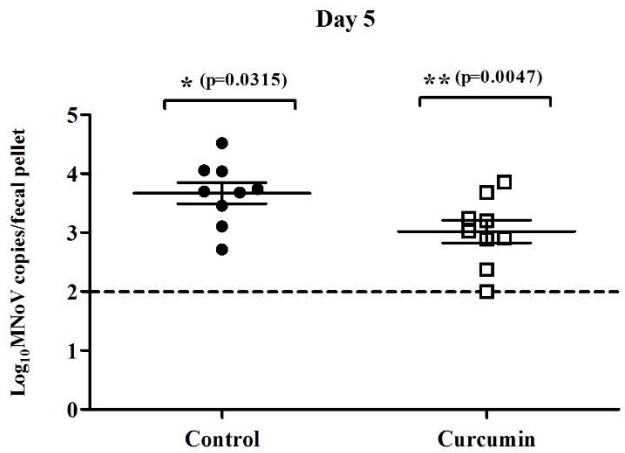

Figure 2. Comparison of MNoV shedding in stool samples of curcumin or vehicle-treated mice.

Mice were inoculated with curcumin everyday from 1 week before infection to 27 DPI. Stool was collected at day 5 after infection and viral loads were determined by real-time RT-PCR. Genome copies were compared between curcumin or vehicle (0.5 % CMC) in MNoV infected mice. N = 7 to 10 mice per each group. The dotted line represents the limit of detection (LOD). Statistical significance was analyzed by Mann-Whitney test. *P<0.05, **P<0.01.

2. Effect of curcumin on MNoV shedding according to time course

To confirm an effect of curcumin on virus shedding according to time course, stool samples were collected at other time points separately. In curcumin group at DPI 13, MNoV genome copies were increased by 3.78 ± 0.19 $\log_{10}$ copies/fecal (Fig.3A). There was no significant difference comparing with control group (3.42 ± 0.10 $\log_{10}$ copies/fecal). MNoV persistently replicated in majority of mice in treated and control group without significant differences at DPI 25.

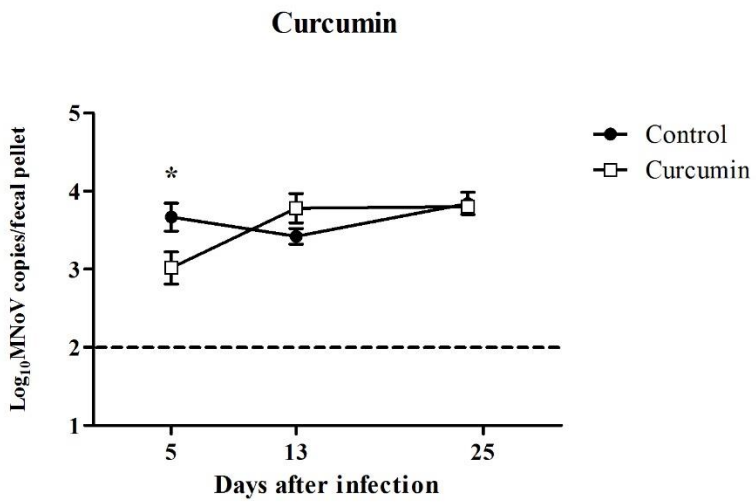

Figure 3. Time course comparison of MNoV shedding shed into fecal pellets.

Curcumin was inoculated to MNoV infected mice everyday for evaluating antiviral effects. Viral loads were analyzed from fecal pellets collected on 5, 13, and 25 DPI. For comparison, genome copies were determined by real-time RT-PCR. Dashed line represents the limit of detection (LOD). Results were analyzed by two-way ANOVA with Bonferroni post-tests. *P<0.05.

3. Effect of curcumin on MNoV replication in tissues

To confirm an effect on MNoV replication on tissues, samples were harvested at DPI 27 and analyzed using real-time PCR. In curcumin group, virus replication was significantly reduced in large intestine 1.29 $\log_{10}$ (*p =0.0206) (Fig.4). Reduction was not observed in small intestine. Tissue specific effect was observed in other tissues. Curcumin inhibited virus replication in MLN (0.43 $\log_{10}$, *p =0.0220) and had no effect in cecum, and peyer's patch (Fig.5).

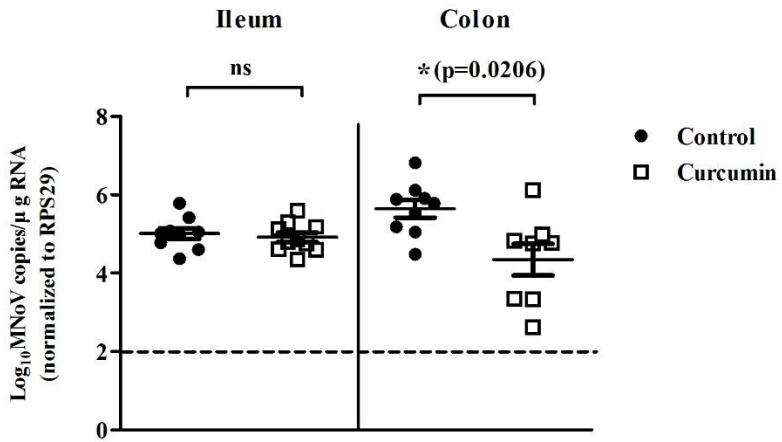

Figure 4. Comparison of MNoV genome copies in intestines of curcumin or vehicle-treated mice.

Tissue samples are harvested in curcumin or vehicle-treated (0.5% CMC) mice at DPI 27. Viral genome copies were evaluated in tissue samples using real-time PCR and normalized with RPS29 (housekeeping gene). Dotted line represents the limit of detection (LOD). Statistical significance was measured by Mann-Whitney test. *P<0.05, **P<0.01. DPI = days post infection; CMC (carboxymethylcellulose).

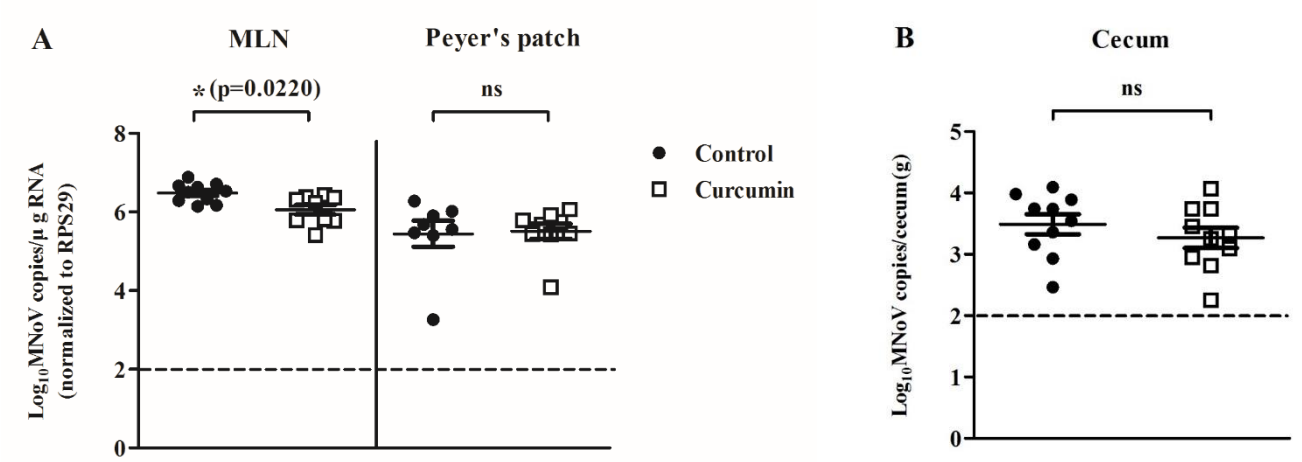

Figure 5. Effects of curcumin on MNoV replication in MLN, peyer's patch, and cecum.

Viral genome copies were evaluated in tissue samples using real-time PCR and normalized with RPS29 (housekeeping gene) at DPI 27. (A) MLN, and peyer's patch (B) Cecum contents were used for quantification. Dotted line represents the limit of detection (LOD). Statistical significance was measured by Mann-Whitney test. *P<0.05. DPI = days post infection

4. Comparison of antiviral gene expression in tissues of curcumin group

To explain tissue-specific inhibition in curcumin group, expression of antiviral cytokines were compared using SYBR green real-time PCR. Interferon-α, -β (Type I interferon), -γ (Type II interferon) were screened in colon, ileum, MLN (Fig.6). IFN-β was significantly promoted in colon and MLN (*p =0.0148 and *p =0.0315) respectively. It had no significant effect in ileum. The regulation of IFN-α also had similar patterns (colon; p =0.0431, MLN; p =0.0947, ileum; p =0.2897). IFN-γ, known as an important activator of macrophages resulting in immune response against bacteria and virus (32) was not promoted in all tissues. Thus, curcumin upregulated type I interferons and its effects were tissue-specific.

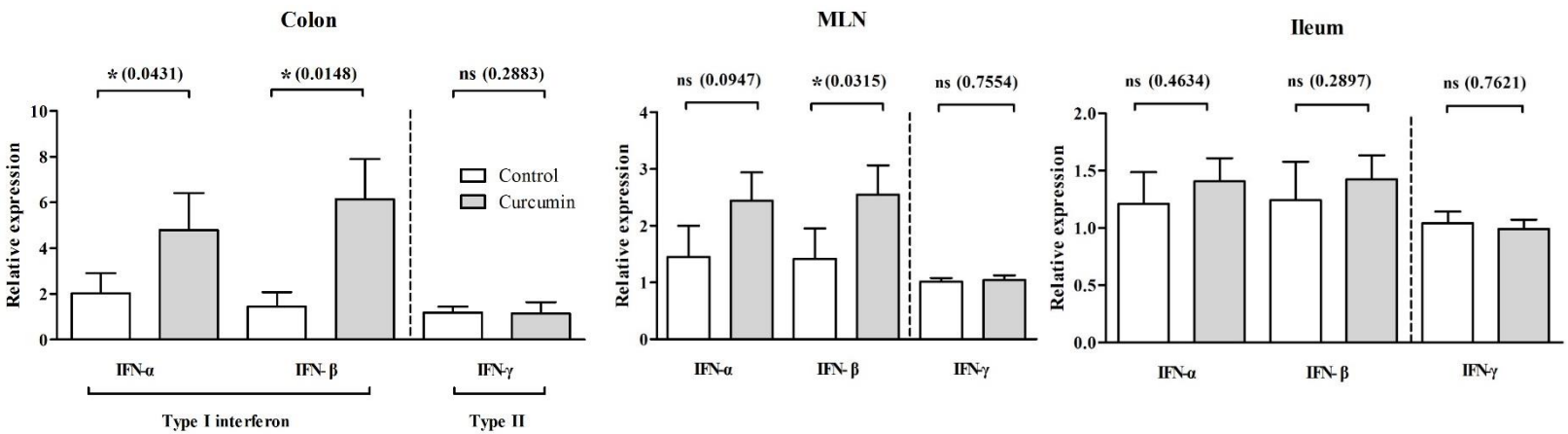

Figure 6. Effects of curcumin on the expression of interferons in tissues of MNoV infected mice.

Interferon (IFN-α, -β, and -γ) expression relative to GAPDH was measured in tissues (colon, MLN, ileum) of curcumin or vehicle-treated (0.5% CMC) mice. Gene expression was measured by SYBR real-time PCR. N= 7 to 10 mice per each group. Statistical significance was measured by Mann Whitney test. *P<0.05.

5. Comparison of microbial diversity changes by curcumin treatment

The microbiome of different tissue sites (ileum, cecum, colon) was analyzed across curcumin treatment. Phylotype diversity of major eight families was compared in Fig. 7 and statistical significance was measured using Mann-Whitney test between curcumin and vehicle treated mouse groups. Major differences were observed in cecum. In curcumin group, *Ruminococcaceae* (**p=0.0089), unclassified *Clostridiales* (***p=0.0002) were significantly increased and *Bacteroidaceae* (***p=0.0005) was decreased comparing with control group. No major differences were observed in colon (*Pseudomonadaceae*; p=0.5490. *Clostridiaceae*; p=0.6842) and ileum.

To visualize the relationship between groups, non-metric multidimensional scaling (NMDS) was performed based on species level OTU data (Fig. 8). Points were plotted closer to each other if they have more similar community and vice versa. Separation between tissues were observed but between treatment were not observed.

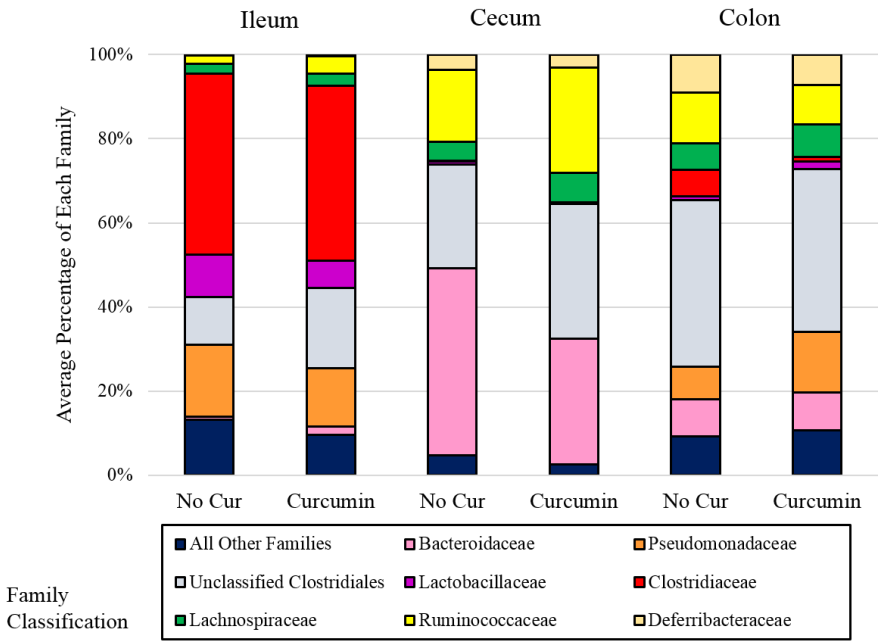

Figure 7. Family level diversity comparisons by curcumin treatment in MNoV infected mice.

Average relative abundance of family level was compared across tissues and curcumin treatment. Relative abundance of each family was averaged for each groups. N=7 to 10 mice per each group.

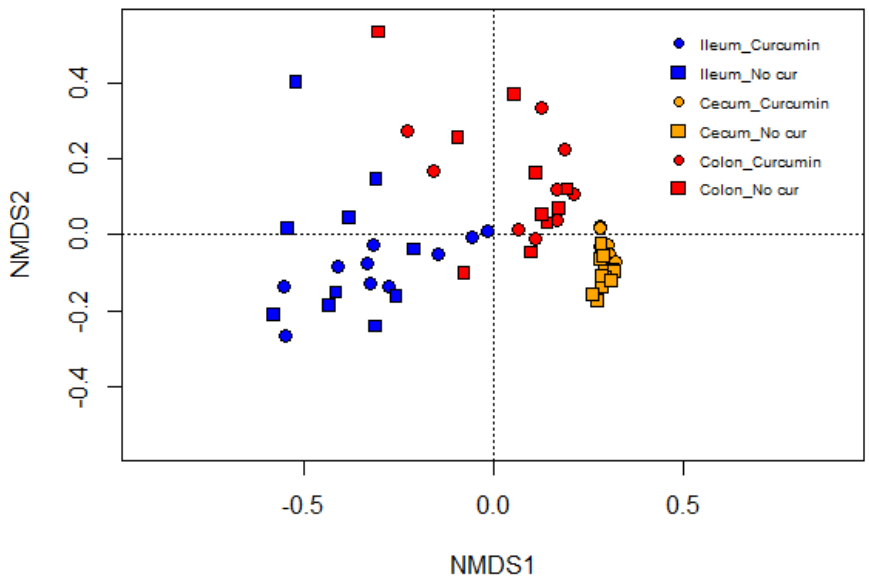

Figure 8. Non-metric multidimensional scaling (NMDS) plot of microbial composition in MNoV infected mice.

The NMDS was generated based on relative operational taxonomic unit (OTU) data of species level. The dissimilarity between samples were measured using Bray-Curtis method. Each symbol represents samples that are colored according to tissues (colon, ileum, and cecum) and shaped according to curcumin treatment.

6. Characterization of specific bacteria change in colon

To find specific microbial change in colon, we applied LDA effect size (LEfSe) method (30) on family level (Fig. 9A). *Mogibacteriaceae* was increased in colon (Fig. 9B). We performed same method to cecum and ileum samples (data not shown). The relative abundance of *Mogibacteriaceae* was significantly increased only in colon (**P=0.0057) (Fig. 10).

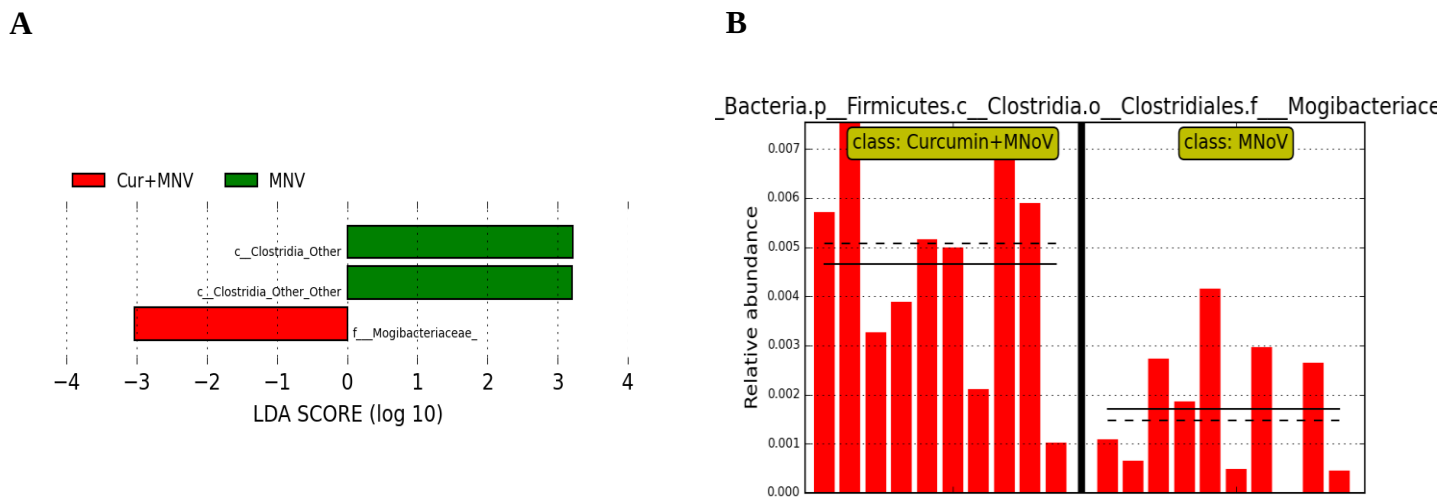

Figure 9. Significant bacterial abundances in colon according to curcumin inoculation in MNoV infected mice.
(A) Characterization of significant abundance changes by curcumin was identified by LEfSe. Statistical analysis was measured using the Kruskal-Wallis test (among classes) and Wilcoxon test (between subclasses) as a p value < 0.05 in both tests. The threshold logarithmic LDA score was set to 3.0. (B)The comparison of Mogibacteriace abundance change was shown separately

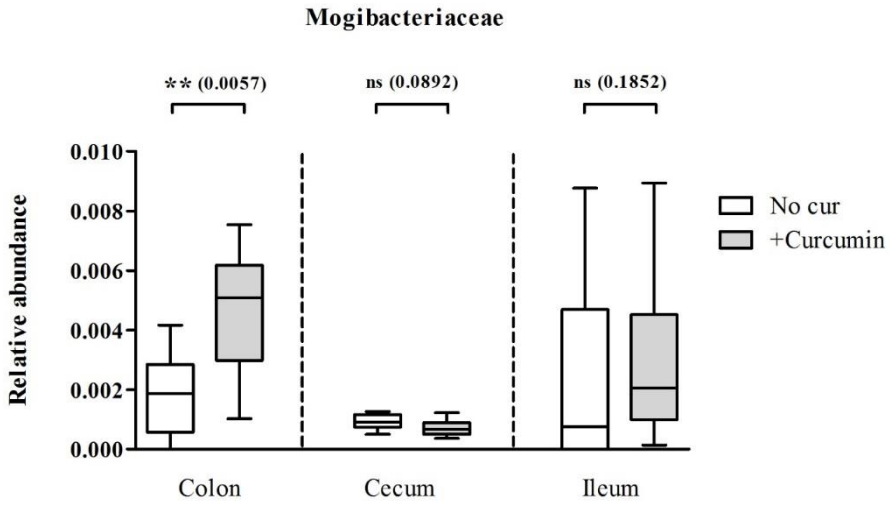

Figure 10. Effects of curcumin to Mogibacteriaceae abundance in MNoV infected mouse tissues.

Box plots were made using species level relative operational taxonomic unit (OTU) data across tissues and curcumin treatment. Whiskers are plotted by Tukey method. Statistical significance was performed using Mann-Whitney test. . *P<0.05, **P<0.01

7. Correlation of Mogibacteriaceae with MNoV

Curcumin inhibited MNoV and increased *Mogibacteriaceae* in only colon. To find out correlation between MNoV virus quantity and abundance *Mogibacteriaceae*, Spearman correlation were performed. The correlation was significant in Spearman method with p=0.0486 and rho=-0.5. Thus, the abundance of the *Mogibacteriaceae* family was weak negative correlation with virus quantity.

IV. Discussion

In this study, curcumin caused an upregulation of antiviral gene expression resulting in inhibition of MNoV replication in colon and mesenteric lymph nodes (MLN). Inhibition was not observed in ileum, peyer's patch (PP), and cecum. Antiviral gene expression which was upregulated in colon and MLN was not observed in ileum. Additionally, we characterized the impact of curcumin on the intestinal microbial community to explain antiviral responses which was restricted to large intestine. We identified the abundance of *Mogibacteriaceae* family was specifically changed in colon. According to Spearman's correlation analysis, the amount of viruses showed negatively weak correlation with *Mogibacteriaceae* family abundance. This study characterizes immunomodulatory roles of curcumin related to antiviral responses and changes in microbial community.

Turmeric, derived from the rhizome of the *Curcuma longa* is a golden spice widely used in Indian culture from the ancient times (11). Curcumin is a polyphenolic compound which gives the yellow color to turmeric. It is called 'the Indian solid gold' for its diverse biological and pharmacological activities such as anti-inflammatory, antioxidant,

antimicrobial, antiviral, and anticancer (10-13, 33). Curcumin also has been reported to regulate various types of immune cell growth and activities (34, 35). Similarly, curcumin is highly safe even at extremely high doses in animal model and human studies (36, 37). Curcumin consumption as a dietary spice ranges up to 100mg/day (38). In clinical test, 12g per day of curcumin is well acceptable (39).

MNoV CR6 replicated persistently along the gastrointestinal (GI) tract and significantly higher in the proximal colon (25). Mesenteric lymph nodes (MLN) was analyzed because MNoV grows in immune cells such as dendritic cells, macrophages, and B cells (27, 40) and also MLN likely reflects the systemic infection. Microfold (M) cell in peyer's patch (PP) is known as a major entry site of pathogens including norovirus (22). In colon and MLN, type I IFN-β was significantly upregulated in these tissues. It is widely known interferons (IFN) are essential to control virus replication on both MNoV and HuNoV (23, 41, 42). Nice et al. reported that C57BL/6 mice infected by MNoV CR6 strain did not induce detectable level of type I IFN mRNAs in MLN, peyer's patch, and colon (25). Thus, curcumin induced type I IFNs in colon and MLN through direct or indirect way resulting in inhibition of MNoV replication. But its effects were specific in those tissues and had no effect on virus

shedding. Nice et al. compared the requirement for IFN receptors in control of fecal shedding (25). Virus shedding was increased in IFN-λ receptor knockout mouse compared with control mouse not IFN-α receptor knockout mouse over 35 days after infection. It means IFN-λ controlled fecal shedding not type I IFNs. Curcumin might not inhibit virus trafficking from lumen to tissues via peyer's patch because MNoV inhibition was not observed in peyer's patch. Thus far, evidences may indicate that curcumin induced immunomodulatory activities related to antiviral effects in large intestines and its effects may be transferred to MLN. It is the first report to identify anti-noroviral effect of curcumin through upregulation of antiviral IFNs.

Additionally, microbiome analysis was performed to explain the inhibition of MNoV restricted to large intestines. The gastrointestinal (GI) tract contains diverse and numerous microbial community with 10^4 - 10^7 bacteria / ml of ileum and 10^{11} - 10^{12} bacteria / ml in colon (43). Therefore, when a virus infiltrates an intestine to infect the host cell, it must interact with the microbiota. The interaction can be beneficial to host or not. Ichinohe et al. found that commensal bacteria activated inflammasome (44) which is required for inactivating influenza virus (45). On the other hand, commensal bacteria may promote viral infection

direct or indirect way. In the case of murine leukemia virus (MuLV), pathogenicity of murine leukemia virus was decreased in germ-free mouse (46). It was identified that commensal bacteria proliferated a lymphoid cell which is target cell by MuLV, thus resulting in more virus replication, and higher leukemia frequency. Polio virus and reovirus utilized bacterial LPS resulting in enhancement on their attachment to host cells (47). Mouse Mammary Tumor Virus (MMTV) evades antiviral response by activation of TLR-4 in a dendritic (DC) cell and it induces IL-10, acting as an immunosuppressive cytokine (48).

In the case of norovirus, enteric bacteria have been reported to serve as a co-factor for norovirus infection (27). Baldridge et al. identified that depletion of enteric bacteria made the MNoV fail to propagate in mouse intestines (26). In recent study, retinoic acid (RA) showed anti-noroviral effect mediated by upregulation of IFN-β (49). In gut microbiome analysis, abundunce of *Lactobacilaceae* family was significantly increased after RA admistration. Interestingly, *Lactobacilaceae* family modulated IFN-β regulation resulting in inhibition of MNoV.

In our study, systemic major bacterial community change was not observed in colon. In LEfSE analysis to discover specific microbial

change, *Mogibacteriaceae* family abundance significantly increased comparing with control group in colon. It was not observed in other tissues to be analyzed. The function of family *Mogibacteriaceae* in the intestine is not much known. *Mogibacteriaceae* were clustered with other microbes that are associated with lean body mass index (BMI) (50) and are associated with the changes of the diet containing cellulose with pectin, xylan in non-obese diabetic (NOD) mouse (51). Further analysis of family *Mogibacteriaceae* is required to know the relationship with MNoV.

This study provided evidences curcumin inhibited MNoV replication mediated by type I IFN. The effects were specific in colon and MLN. Also, curcumin was shown to change the gut microbiota. *Mogibacteriaceae* family was significantly changed in colon. This changes were not observed in other tissues (cecum, ileum). Relative abundance of *Mogibacteriaceae* has weak negative correlation with the amount of virus. Future studies should focus on the effect of *Mogibacteriaceae* against MNoV replication and what mechanisms are involved in that interaction.

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국문초록

Curcumin을 이용한 항노로바이러스 효과 및 기전연구

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노로바이러스 감염으로 인한 장염발생은 전세계적으로 비세균성 위장염의 90%를 차지한다. 감염으로 인한 증상과 잠복기는 24-48시간으로 알려져 있으나, 면역력이 저하된 환자에게서 노로바이러스가 만성적으로 감염을 일으키는 것으로 보고 되어있다. 이뿐 아니라 만성적으로 감염된 환자의 숙주 내에서 노로바이러스의 변이가 일어나 또 다른 노로바이러스 집단 감염을 일으킬 수 있다. 현재 상용화 되어 있는 치료제가 없기 때문에, 노로바이러스를 제어할 수 있는 안전하고 접근이 용이한 치료제의 개발이 필요하다. 식물과 영양소의 합성어이며 식물이 가지고 있는 영양소를 의미하는 phytonutrient 는 예로부터 면역기능과 감염병에 대한 저항력 증가 효과가 알려져 왔고, 이로 인해 전통적인 약제에 첨가되어 사용되어져 왔다. 본 실험에서는 강황의 유효성분인 커큐민의 노로바이러스에 대한 감염 저해능을 동물 모델을 이용하여 평가하였다. 위의 물질을 매일 쥐에게 지속적으로 투여하고 쥐노로바이러스 (murine norovirus CR6 strain) 를 경구를 통하여 감염시켰다. 분변과 조직에서의 바이러스 양은 실시간 중합효소 연쇄 반응법 (realime PCR)을 이용하여 정량하였다. 그 결과, 커큐민이 결장(colon)과 장간막 림프절(mesenteric lymph nodes)에서 바이러스의 증식을 저해한 것을 발견하였고, 위의 조직에서 항바이러스성 인터페론 (type I interferon)이 유의하게 증가하였음을 발견하였다. 그러나 바이러스의 저감은 회장(ileum)과 페이에르판(peyer's patch)에서는 발견되지 않았다. 이러한 현상의 원인을 장내미생물총과의 관계로 가정하고, 16S rRNA 시퀀싱을 이용하여 장내미생물총을 분석하여 비교하였다. 분석 결과 결장속에서 *Mogibacteriaceae* 의 존재비 (abundance)가 다른 조직과 달리 유의하게

증가하였음을 확인하였고, 바이러스 양과의 상관관계 분석에서 약한 음의 상관관계를 보임을 확인할 수 있었다. 본 실험을 통하여 커큐민이 결장에서 항바이러스성 인터페론을 증가시킴을 확인하였고, 이로 인해 쥐노로바이러스의 양이 감소하였음을 확인하였다. 또한 특정한 세균이 노로바이러스의 제어와 상관관계가 있음을 확인할 수 있었다.

주요 단어: 커큐민, 쥐노로바이러스, 만성감염, 미생물총

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